

## FINDING OF NO SIGNIFICANT IMPACT

### USAMRMC-FUNDED RESEARCH AT LOUISIANA STATE UNIVERSITY MEDICAL CENTER ENVIRONMENTAL ASSESSMENT

- 1. PROPOSED ACTION:** The proposed action (Alternative I, preferred alternative) and subject of this Environmental Assessment (EA) is the conduct of a proposed research project for the U.S. Army Medical Research and Materiel Command (USAMRMC) at the Louisiana State University Medical Center (LSUMC). The proposed study is a component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats. The research project – *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* – will be conducted at LSUMC, Shreveport, Louisiana. *Brucella* is a potential biological warfare threat for which there is currently no acceptable human vaccine. Researchers will prepare and investigate strains of *Brucella melitensis*, one of the causative agents of human brucellosis, for their potential for use in vaccines.
- 2. ALTERNATIVES CONSIDERED:** During the preparation of this EA, one alternative to the proposed action was identified. This alternative is to cease funding of the research proposed by LSUMC (Alternative II, no action).
- 3. ENVIRONMENTAL CONSEQUENCES AND MITIGATION MEASURES:** It is unlikely that significant adverse environmental impacts will result from implementing the proposed action. The preferred alternative includes adherence to existing regulations and standards and the use of specialized facilities. Adherence to health, safety, and environmental regulations applicable to the conduct of research involving biohazardous microorganisms will mitigate risk to the workforce and ensure environmental protection.
- 4. FACTORS CONSIDERED IN THE FINDING OF NO SIGNIFICANT IMPACT:** The EA systematically reviews the nature of the proposed action and associated risks and issues. Particular attention is given to protection of the workforce and surrounding community. Alternatives with regard to needs of the United States and the U.S. Army and potential adverse effects on the environment are evaluated.
- 5. CONCLUSIONS:** The principal conclusions of this EA are: (1) the conduct of the proposed research project – *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* – (Alternative I, preferred alternative) is not expected to result in significant adverse environmental impact; (2) implementing the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an effective vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II, no action) will eliminate the negligible environmental impacts associated with conducting the research, but it will also eliminate potentially significant advances in developing a human brucellosis vaccine.

FOR THE COMMANDER

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Comments on this Finding of No Significant Impact may be directed to COMMANDER, USAMRMC, ATTN: MCMR-PA, Charles Dasey, Fort Detrick, MD 21702-5012 and must be received by August 17, 1998. Copies of the EA are available for review by the public at the Shreve Memorial Library, 424 Texas Street, Shreveport, LA 71120, and at: <http://MRMC-www.army.mil>. Copies may also be obtained by writing to COMMANDER, USAMRMC, ATTN: MCMR-RCQ-E (Dr. Robert J. Carton), Fort Detrick, MD 21702-5012.

**ENVIRONMENTAL ASSESSMENT  
of  
USAMRMC-Funded Research  
at Louisiana State University Medical Center**

**Identification of Secondary Mutations Which Enhance and Stabilize the  
Attenuation of *Brucella htrA* Mutants: Improving *Brucella htrA*-based Strains  
as Vaccine Candidates**

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## EXECUTIVE SUMMARY

This Environmental Assessment (EA) was prepared in accordance with guidance provided in Army Regulation (AR) 200-2, *Environmental Effects of Army Actions*, dated December 23, 1988, implementing the National Environmental Policy Act (NEPA) (42 USC 4321-4347). This EA, *Environmental Assessment of U.S. Army Medical Research and Materiel Command (USAMRMC)-Funded Research at Louisiana State University Medical Center*, was prepared by USAMRMC with assistance from Science Applications International Corporation (SAIC) under Contract Number DAMD17-93-C-3141.

This EA describes and analyzes the potential adverse environmental impacts, including human health impacts, associated with conducting a proposed research project funded by the USAMRMC. The proposed study is a necessary component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats. The proposed research was submitted to the USAMRMC in response to a Broad Agency Announcement solicitation. The proposed research – *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* – will be conducted at Louisiana State University Medical Center (LSUMC). Researchers will prepare and investigate strains of *Brucella melitensis*, one of the bacteria that cause human brucellosis. *Brucella* is considered a potential biological warfare threat. There is currently no acceptable vaccine for human brucellosis.

During the preparation of this EA, one alternative to the proposed action was identified. This alternative is to cease funding of the research as proposed by LSUMC (Alternative II, no action). This EA characterizes the probable environmental impacts, including impacts to human health, that might result from conducting either the proposed research (Alternative I, the preferred alternative) or the alternative considered.

The principal conclusions of this EA are: (1) the conduct of the proposed research project– *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* (Alternative I, the preferred alternative) is not expected to result in significant adverse environmental impacts; (2) implementation of the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an acceptable vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II, no action) will eliminate the negligible environmental impacts associated with conducting the research, but it will also eliminate potentially significant advances in human brucellosis vaccine development.

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## 1.0 PURPOSE AND NEED FOR THE PROPOSED ACTION

This EA describes the potential adverse environmental impacts, including human health impacts, associated with conducting a proposed research project to be funded by USAMRMC. The proposed research was submitted to the USAMRMC in response to a Broad Agency Announcement solicitation. The proposed study – *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* – will be conducted at LSUMC and is described in Section 2.0. This analysis considers impacts expected from conducting the proposed research, cumulative impacts that might occur after several years, and impacts resulting from an accident or incident. One alternative to the proposed action is also discussed (see Sections 3 and 5).

The proposed research study is a necessary component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats. The USAMRMC was established in 1994 as a major subordinate command of the U.S. Army Medical Command (MEDCOM) and is the lead agency for the Department of Defense (DoD) Biological Defense Research Program (BDRP). Research and development activities in support of the BDRP are conducted at military research facilities and through contracts and Cooperative Research and Development Agreements with universities, other institutions, and industry. These programs are directed and monitored by USAMRMC headquarters staff officers from grant award through completion.

The bacterium *Brucella*, the causative agent of brucellosis, has been identified as a potential biological warfare threat. Currently, there is no effective, acceptable vaccine for human brucellosis. The objective of the proposed research project is to study and develop *Brucella* strains with potential use in human vaccines (see Section 2.3). Mice will be immunized with *Brucella* strains genetically engineered to be less virulent while retaining the ability to induce a protective immune response. Immunized mice will then be challenged with disease-causing *Brucella* organisms to determine the effectiveness of the immunization in preventing disease. This study is viewed as a necessary component of USAMRMC efforts to develop medical countermeasures against potential biological warfare threats.

The National Environmental Policy Act (NEPA) (42 U.S. Code [USC] 4321-4347) requires that each Federal agency consider the potential environmental impacts associated with proposed major actions. The Council on Environmental Quality (CEQ), Executive Office of the President, has promulgated regulations implementing NEPA (40 Code of Federal Regulations [CFR] Parts 1500-1508). AR 200-2, *Environmental Effects of Army Actions*, dated December 23, 1988 (32 CFR 651), is the Department of the Army's (DA) implementation of NEPA and the CEQ regulations. USAMRMC environmental policy requires that proposed actions involving the operation of biosafety level (BSL)-3/BSL-4 laboratories undergo environmental assessment in accordance with CEQ regulations and AR 200-2. This EA was prepared in accordance with AR 200-2 and CEQ regulations.

Programmatic aspects of the BDRP were previously evaluated within the context of NEPA. The BDRP Final Programmatic Environmental Impact Statement (FPEIS) was prepared by the DoD in 1989 to examine the possible and probable environmental impacts of BDRP activities. The Record of Decision (ROD) resulting from the BDRP FPEIS found that although certain aspects

of the program were controversial, such as aerosol testing and the use of genetically engineered microorganisms (GEMs), there was no evidence of any significant environmental impact. Various public and government groups were involved with preparing the BDRP FPEIS. Dialogues and analyses indicated that public concerns were programmatic in nature and not directly related to actual environmental impacts at specific sites within the BDRP. The analyses found that any potential adverse environmental impacts to the human environment associated with the continuation of BDRP research efforts were minimal. In this EA, BDRP activities, funded by the USAMRMC and performed at LSUMC, are examined for their potential to cause significant adverse environmental impacts.

## **2.0 DESCRIPTION OF THE PROPOSED ACTION**

### **2.1 INTRODUCTION**

The bacterium *Brucella* is the causative agent of human brucellosis, also referred to as Bang's disease, undulant fever, Malta fever, or Mediterranean fever. Brucellosis in humans is caused by one of five *Brucella* species and its distribution is worldwide. *Brucella* also causes disease in animals and can be transmitted from animals to humans. Brucellosis is rare in the U.S. because food sanitation and effective animal brucellosis control programs prevent its occurrence and transmission. Although a brucellosis vaccine is available for animals, there are currently no acceptable vaccines for humans. Treatment for infected humans involves administering antibiotics immediately following suspected exposure. Symptoms of brucellosis may include fever, chills, night sweats, headache, muscle and joint pain, and profound fatigue. Disease symptoms may become evident within days of exposure or may develop gradually. Untreated, brucellosis rarely causes death, but may it result in chronic debilitating disease symptoms and complications.

Humans acquire brucellosis by consuming *Brucella*-contaminated animal products or by coming into contact with diseased animals or their secretions. In rare cases, brucellosis may be transmitted from close personal contact. *Brucella* is highly infectious, and human brucellosis can result from exposure to as few as 10 *Brucella* organisms (Harding and Liberman, 1995; Kaufmann, 1995; Kaufmann and Boyce, 1995). In fact, laboratory-acquired infections have occurred from entry of the organism through microscopic breaks in the skin; accidental sticks with contaminated objects; inhaling organisms from contaminated air; direct contact of contaminated materials with mucous membranes of the nose or eye; or accidental ingestion (Centers for Disease Control and Prevention [CDC]/National Institutes of Health [NIH], 1993; Harding and Liberman, 1995; Sewell, 1995).

The proposed research is described in Section 2.3. As with other potentially disease-causing microorganisms, work with *Brucella* requires the application of special work practices and engineering controls to protect worker and public health and safety as well as the integrity of research findings. Safety practices and procedures are discussed in Section 2.4.

### **2.2 ORGANIZATION, LOCATION, AND FACILITIES**

LSUMC is located on Kings Highway in the city of Shreveport, Caddo Parish, Louisiana. Facilities at LSUMC include the Louisiana State University Hospital Shreveport, the Medical Library, and the LSU School of Medicine. The Virginia K. Shehee Biomedical Research Institute is connected to the LSU School of Medicine (Biomedical Research Foundation of Northwest Louisiana, 1996).

Scientists in the Department of Microbiology and Immunology at LSUMC will conduct the proposed research at the Biomedical Research Institute. The 160,000-square-foot building was constructed in 1995. It includes a 550-square-foot BSL-3 containment suite and animal care facilities. A BSL-2 laboratory located in the Department of Microbiology and Immunology in Building B will also be used. A more detailed description of the environmental setting of LSUMC is provided in Section 4.0.

## 2.3 PROPOSED STUDY ACTIVITIES

The proposed research will use the BALB/c mouse as an experimental model since both mice and humans develop chronic forms of *Brucella* infection. *Brucella* species are usually found in white blood cells. *Brucella* infect macrophages (specialized white blood cells that ingest and digest foreign particles). It is desirable to identify bacterial genes that allow *Brucella* to establish infection and to target those genes for deletion or disruption. Evidence suggests that the primary method used by host cells to kill intracellular *Brucella* is reactive oxygen intermediate-mediated killing. The proposed research will target specific genes that protect *Brucella* from oxidative killing by host cells.

Desirable traits in a vaccine candidate include limited replication in the host and induction of a strong cellular immune response. In mice, *Brucella abortus* and *Brucella melitensis* *htrA* mutants PHE1 and RWP5 are susceptible to reactive oxygen intermediate-mediated killing but are only transiently attenuated. These mutants cannot be tested as vaccine candidates in humans because of the potential to cause illness or disease. The investigators plan to introduce secondary mutations into the *Brucella htrA* mutants that will enhance and stabilize attenuation in the mouse model as well as further compromise resistance to reactive oxygen-mediated killing. The first mutations to be used will be *katE* and *sodC*. The second type will be a *prc* deletion mutation.

ColE1-based plasmids will be used to introduce mutations into *Brucella* species because they do not replicate in the bacteria. Internal coding regions in the targeted genes will be replaced with a chloramphenicol (antibiotic) resistance gene to construct gene replacement vectors. Once constructed, these gene replacement vectors will be introduced into *Brucella* strains through electroporation. The mutants will be selected on Schaedler agar supplemented with 5% defibrinated blood (SBA) containing chloramphenicol (SBAC). The purported double mutants will then be selected on SBAC with kanamycin (antibiotic) (SBACk) and screened for sensitivity to ampicillin by plating on SBA supplemented with ampicillin (SBAA). The genotypes of the mutants will be analyzed by Southern blot using specific probes. Western blot analysis with specific antisera will be used to verify that the mutants fail to produce HtrA, KatE, SodC, or the *prc* gene product.

The double mutants will be evaluated and compared to their respective parental strains. Modified disk sensitivity assays will be performed to assess some mutants' sensitivity to killing by reactive oxygen intermediates and reactive nitrogen intermediates. Mutants constructed from *Brucella melitensis* strains 16M and RWP5 will be evaluated in liquid media, because RWP5 will grow only on Schaedler agar supplemented with blood. These experiments will use Gerhardt's Minimal Medium and disks saturated with solutions of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) solution, the superoxide radical (O<sub>2</sub><sup>-</sup>) generator plumbagin, hypochlorous acid (HOCl), and the nitric oxide (NO) generator diethylenetriamine-NO adduct. If other mutants unable to grow on Schaedler agar are identified, they will also be evaluated in liquid media.

The double mutants will be assessed for their intracellular survival and replication in the presence of cultured BALB/c mouse phagocytes (neutrophils and macrophages). In one set of experiments, the resistance of the double mutants to killing by neutrophils will be tested. The percentage ( $\geq 89\%$ ) of neutrophils in cell samples obtained by lavage of the peritoneal cavities of

euthanized 8- to 12-week-old female BALB/c mice will be determined by Wright Giemsa stain. The washed cells will be plated into 96 well tissue culture plates. *Brucella* unopsonized or opsonized with 10% normal BALB/c mouse serum will be added to each well. After incubation with 5% carbon dioxide (during which phagocytosis occurs), the neutrophils will be lysed with deoxycholate and the number of surviving *Brucella* will be determined by serial dilution and plating on SBA. Reactive oxygen intermediate production by the cultured neutrophils will be monitored.

The resistance of the double mutants to killing by mouse macrophages will be tested in another set of experiments. Macrophages from the peritoneal cavities of 8- to 12-week-old euthanized mice will be incubated in 96 well tissue culture plates. After nonadherent cells are washed away to enrich the cell cultures, unopsonized or opsonized *Brucella* will be added. After allowing phagocytosis to proceed, the culture medium will be replaced with medium to which gentamicin (antibiotic) has been added, and incubation will continue. After washing, the macrophages will be lysed with deoxycholate. The number of *Brucella* which survived intracellularly (in the macrophages) will be determined by serial dilution and plating. The investigators will also conduct experiments to determine the effect of activation of the macrophages on intracellular killing of *Brucella*. Interferon gamma will be added to cell cultures to activate the macrophages prior to adding *Brucella*. Reactive oxygen intermediate production by the cultured macrophages will be monitored. If a mutant exhibits significantly increased susceptibility to intracellular killing relative to its parental strain, additional assays will be performed employing reactive oxygen and nitrogen intermediate specific quenchers to verify the role of these compounds in the differential killing of the mutants by host phagocytes. Compounds that may be used include catalase, superoxide dismutase, mannitol, methionine, or N<sup>G</sup>-monomethyl-L-arginine. The killing of mutants by a (mouse) monocyte/macrophage cell line defective in reactive oxygen intermediate production will be assessed by similar cell culture methods.

The double mutants' attenuation and ability to induce an immune response in BALB/c mice will be studied. *Brucella* strains diluted in sterile phosphate-buffered saline to concentrations of about  $5 \times 10^4$  colony forming units (cfu) will be injected intraperitoneally into mice. Nine groups of five female BALB/c mice about 10 weeks of age will be inoculated for each strain tested. At specific intervals, 1 to 20 weeks after infection, mice from each group will be euthanized by halothane overdose. After blood samples are obtained, the spleens and livers will be cultured. Tests will be performed to confirm the identity of the reisolates. The genetic stability of the strains will be verified using polymerase chain reaction methods. The serum obtained from the mice will be frozen. Enzyme-linked immunosorbent assay (ELISA) will be used to assess the induction of *Brucella*-specific antibody responses in the infected mice. The induction of cellular immunity in the mice will be evaluated by injecting *Brucella* cell lysate into the right hind foot pad 48 hours before euthanasia. Phosphate-buffered saline will be injected into the left hind foot pad as a control. After euthanasia, histopathologic evaluation of inflammatory changes will be performed on the specimens.

The investigators anticipate that some of the double mutants will be cleared from the spleens and livers of the infected mice by 6 weeks after inoculation. It is desirable to identify those mutants that exhibit the ability to induce protective immunity by accelerated clearance. Protection is defined as a significant decrease in tissue colonization in the vaccinated mice compared to the

nonvaccinated mice. Mice will be inoculated with  $5 \times 10^4$  cfu of double mutants. Groups of mice will be injected with phosphate-buffered saline (negative control) and similar concentrations of *Brucella abortus* strain 19 and *Brucella melitensis* Rev 1 (positive controls). Cross-protection (between strains) will be evaluated. After 6 weeks, the mice will be challenged with  $5 \times 10^4$  cfu of a virulent parental strain. The mice will be euthanized 1 week after the challenge. Their livers and spleens will be cultured, blood will be obtained and stored, and histopathologic evaluation of the hind feet will be performed to evaluate inflammatory changes. Any immunization protocols showing significant protection at 1 week will be repeated, and protection at 4 weeks after challenge will be evaluated (LSUMC, 1997).

An estimated 4,120 BALB/c mice will be used over the 4-year span of the proposed research. Six personnel will work in the BSL-3 suite. The six personnel will work with *Brucella* species and *Escherichia coli* (Roop, 1998a).

## **2.4 SAFETY**

The proposed research requires the use of materials that require special handling to mitigate potential risks to human health and the environment. These materials include *Brucella abortus* and *Brucella melitensis*. In addition, the proposed research involves the use of recombinant deoxyribonucleic acid (DNA) molecules, chemicals, and radioisotopes. A Facility Safety Plan detailing the environmental, safety, and occupational health policies and programs under which the research will be conducted has been submitted to and approved by USAMRMC.

### **2.4.1 Biological Safety**

Both LSUMC and the DA require adherence to the guidelines for biological safety described in *Biosafety in Microbiological and Biomedical Laboratories* (CDC/NIH, 1993). These guidelines recommend the laboratory practices, techniques, facilities, and equipment necessary to contain infectious organisms of varying degrees of pathogenicity and virulence and their products. These measures have been developed to minimize risks to human health and the environment. Regardless of location, research funded by the DA and involving biological defense agents such as *Brucella* must also meet the safety requirements detailed in 32 CFR Parts 626 (*Biological Defense Safety Program*, AR 385-69) and 627 (*The Biological Safety Program; Technical Safety Requirements*, DA Pamphlet 385-69). These regulations require implementation of the CDC/NIH guidelines.

The CDC/NIH guidelines describe four BSLs established for conducting laboratory operations with infectious agents and/or their toxins. BSL-1 practices, safety equipment, and facilities are appropriate for facilities in which work involves defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. BSL-2 practices, safety equipment, and facilities are appropriate for facilities in which work involves the broad spectrum of indigenous (native) moderate-risk agents present in the community and associated with human disease of varying severity. Work with indigenous or exotic agents that have serious or lethal consequences if inhaled requires BSL-3 containment. BSL-4 practices, safety equipment, and facilities are required for work with dangerous and exotic agents posing a high individual risk of life-threatening disease. The CDC/NIH guidelines include agent summary statements that provide

specific information on laboratory hazards associated with various agents and guidance for selecting appropriate BSLs. Under the CDC/NIH guidelines, the laboratory director is responsible for determining the appropriate BSL based upon “the virulence, pathogenicity, biological stability, route of spread, and communicability of the agent; the nature or function of the laboratory; the procedures and manipulations involving the agent; the endemicity of the agent; and the availability of effective vaccines or therapeutic measures (CDC/NIH, 1993).”

In accordance with CDC/NIH guidelines and 32 CFR Parts 626 and 627, it has been determined that the proposed research requires BSL-2 practices and facilities for work with recombinant technology and *Escherichia coli* and BSL-3 practices and facilities for work involving *Brucella*. BSL-3 “differs from BSL-2 in that (1) more extensive training in handling pathogenic and potentially lethal agents is necessary for laboratory personnel; (2) all procedures involving the manipulation of infectious material are conducted within biological safety cabinets, other physical containment devices, or by personnel wearing appropriate personal protective clothing devices; [and] (3) the laboratory has special engineering and design features, including access zones, sealed penetrations, and directional airflow (32 CFR 627).”

Laboratory work involving animal challenges, *Brucella* cultivation, and DNA extractions is conducted in the BSL-3 suite and all work with biohazardous agents must be performed in a Class II biological safety cabinet. At LSUMC, research involving the use of *Brucella* is conducted in the BSL-3 facilities located on the 9<sup>th</sup> floor of the Biomedical Research Institute. Standard operating procedures (SOPs) for work conducted in the BSL-3 laboratory at LSUMC are found in the *LSUMC Biosafety Level 3 Laboratory Protocol*.

BSL-3 facilities must have signs posted on all doors to indicate their BSL-3 designation, the agent(s) in use within, and the individuals to contact in the event of an emergency. In addition, measures are required to limit and control access to BSL-3 laboratories. Key cards permit monitored access to the Biomedical Research Institute, its elevators, and containment laboratories. The Project Director is responsible for providing training and instruction in laboratory procedures. Documentation of training is recorded in the BSL3 Safety Log, and affidavits pertaining to instruction are kept on file. All workers must read and sign the SOPs in the BSL-2 and BSL-3 Biosafety Manuals. Emergency equipment, including fire-suppression systems, emergency call button, and eyewash stations, is located within the BSL-3 suite. The BSL-3 laboratory must be secured with a lock at all times and the doors magnetically controlled to prevent unauthorized entry. Workers enter the BSL-3 hallway via an anteroom. The anteroom functions as an airlock and includes a sprinkler system for fire suppression, double-door autoclave, emergency shower, motion-activated handwash system in the sink, and personal protective equipment (including Tyvek® coveralls). In addition to entry limitations, the BSL-3 laboratory is kept at negative (lower) air pressure relative to adjacent areas. Negative airflow is directional from the hallway into each room in the BSL-3 suite; the net flow of air is into, not out of, the BSL-3 suite. Surfaces within the anteroom, laboratories, and the adjacent hallway are sealed with epoxy paint and all penetrations to the room are sealed. BSL-3 laboratory freezers and refrigerators containing stock cultures must be labeled with biohazard signs. Potentially contaminated work materials are not removed from the BSL-3 facility until they are rendered noninfectious by chemical disinfectant or autoclave. The autoclave is located between the hallway and connecting anteroom, further limiting the movement of potentially contaminated materials.

The BSL-2 laboratory is locked when not in use; during use, access is restricted to authorized personnel directly involved in research. Workers must wear personal protective equipment, and a chemical spill kit, fire extinguisher, and eyewash station must be available in the room. Work is performed within a Class IIA Type B3 biological safety cabinet that provides directional airflow and filters exhaust using high-efficiency particulate air (HEPA) filtration. Freezers and refrigerators used to store stock cultures of *Brucella* strains must be labeled with biohazard signs. An emergency shower is located just outside the BSL-2 laboratory entrance.

In addition to safety requirements related to the use of *Brucella*, the proposed research also requires adherence to standards and procedures for the safe use of recombinant DNA molecules. Recombinant DNA molecules are defined as either “molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell” or “molecules that result from the replication of those described above.” The *NIH Guidelines for Research Involving Recombinant DNA Molecules* (NIH, 1997) specify practices for constructing and handling recombinant DNA molecules and the organisms or viruses containing recombinant DNA molecules. The guidelines specify procedures for authorizing and overseeing such work, laboratory facilities, and work practice controls. In addition, the guidelines classify agents according to risk and establish procedures for institutional oversight.

*Brucella* is classified by the NIH guidelines as a Class 3 agent. Work involving recombinant DNA procedures on Class 3 agents must be registered with an Institutional Biosafety Committee (IBC). The LSUMC IBC has approved the procedures to be used in the proposed research in accordance with the NIH guidelines. Using those guidelines, the LSUMC IBC must review all research involving biological materials to verify compliance with the guidelines and Federal regulations related to handling of biological materials. LSUMC is registered with the CDC (Certification No. 19970823-492) under the provisions of the *Select Agent Transfer Tracking System* (42 CFR Part 72.6), which regulates storage and shipping of select pathogens and toxic materials that may be considered potential agents of biological warfare (LSUMC, 1997).

At LSUMC, the BSL-3 Safety Committee meets quarterly to discuss and evaluate the safety efforts, incidents, procedures, and practices used in the BSL-3 laboratories. Committee members include the LSUMC Safety Officer, the chairman of the LSUMC IBC, the Project Director, the Laboratory Director, and research and animal care personnel. A record of the meetings is maintained in the Safety Log.

The *LSUMC Biosafety Level 3 (BL3) Laboratory Protocol* and the *LSUMC Biosafety Level 2 (BL2) Laboratory Protocol* contain guidelines for conducting research using agents requiring, respectively, BSL-3 and BSL-2 containment and are reviewed annually. The designated Project and Laboratory Directors are responsible for ensuring adherence to BSL-3 guidelines. The Laboratory Director approves access to the BSL-3 suite only after personnel complete the required instruction and training. Laboratory personnel at LSUMC must conduct daily safety audits of the BSL-3 suite using the 32 CFR Part 627, *Laboratory Safety Inspection Checklist*, as a guide. The Project Director conducts a weekly safety audit, and a representative from the LSUMC Safety Office conducts a monthly safety audit. All results of safety audits and inspections are recorded in the BSL-3 Safety Log. The Safety Log also contains the results of the BSL-2 laboratory quarterly inspections by the LSUMC Department of Environmental Health and

Safety. USAMRMC personnel perform an annual safety inspection of all containment facilities. A USAMRMC inspection of the BSL-2 and BSL-3 laboratories, animal facilities, and support facilities was conducted on May 6, 1998 using the *Basic Checklist for Biosafety Levels 1, 2, and 3* (DA Pamphlet 385-69) as a guideline. The LSUMC facilities and procedures were in compliance with USAMRMC requirements. The USAMRMC biosafety officer recommended sustaining the existing LSUMC operations and laboratory procedures (Hawley, 1998).

#### **2.4.2 Chemical Safety**

Hazardous chemicals that will be used in the conduct of the proposed research include: ethidium bromide, acrylamide, sodium hydroxide, trichloroacetic acid, hydrochloric acid, acetic acid, butanol, methanol, 2-propanol, acetone, ethanol, formaldehyde, xylene, phenol, and chloroform (LSUMC, 1997). The handling and use of hazardous chemicals is regulated by Occupational Safety and Health Administration (OSHA) regulations (29 CFR Part 1910.1450, *Occupational Exposure to Chemicals in Laboratories*). The LSUMC Office of Environmental Health and Safety is responsible for safety programs pertaining to the use of biological materials, chemicals, and radioisotopes. The Office of Environmental Health and Safety has prepared a Chemical Hygiene Plan (CHP) and oversees its implementation. The LSUMC Chemical Safety Manual contains the CHP and information pertaining to emergency notification, community “Right-to-Know,” chemical handling, fume hoods, spill response, and bloodborne pathogen exposure control. OSHA regulations require training for all personnel prior to work assignments on new tasks with the potential for exposure to hazardous chemicals. Information and training continue through occasional refresher courses. Training includes information about accessing Material Safety Data Sheets (MSDSs). The LSUMC CHP and laboratory-specific documents provide information about handling controlled substances, chemical acquisition, chemical storage, potential health risks, environmental monitoring, personal protective equipment, use of fume hoods, safety procedures, inspections, and laboratory audits. The Chemical Hygiene Office inspects institutional laboratories on a semiannual basis. For information about chemical waste handling and disposal, see Section 2.6.

#### **2.4.3 Radiologic Safety**

Radioisotopes needed for the proposed research will be used in the BSL-2 facility and include  $^{32}\text{P}$  (phosphorus) and  $^{35}\text{S}$  (sulfur). The U.S. Nuclear Regulatory Commission (NRC) regulates the use of radioisotopes. The proposed research will be performed under NRC license number LA-001-L01 (expiration date May 31, 1999). NRC regulations require the preparation of written guidelines detailing the safe storage and handling of radiologic materials. The LSUMC Institutional Radiation Safety Committee reviews and approves all work requiring the use of radioisotopes. The Institutional Radiation Safety Officer maintains inventories and records of radioisotope use. Laboratory personnel must monitor the radioactivity level monthly and record the results in a notebook kept in the laboratory. The Radiation Safety Office must monitor the radioactivity level in laboratory on a quarterly basis. The results must be recorded in the BSL-2 laboratory notebook and filed at the Radiation Safety Office (LSUMC, 1998a). For information about radiologic waste handling and disposal, see Section 2.6.

## 2.5 SECURITY

The Biomedical Research Institute and the BSL-3 laboratory are accessible only by key card. Only workers involved directly in the research are permitted access to the BSL-3 biocontainment suite, and only after completion of instruction and training by authorized personnel. The BSL-2 laboratory is locked when not in use and access is restricted to authorized personnel.

## 2.6 WASTE STREAM MANAGEMENT

It is estimated that the proposed research will generate 1,120 pounds of solid wastes annually. Included in this estimate are regulated wastes such as sharps (20 pounds), potentially contaminated materials (150 pounds), animal wastes (650 pounds), as well as general solid waste (300 pounds). It is estimated that 1,000 gallons (gal) of wastewater and 10 gal of liquid infectious wastes will be generated annually (Roop, 1998a).

All wastes contaminated or potentially contaminated with infectious material must be rendered noninfectious before removal from the laboratory suite for disposal. This decontamination is accomplished by a combination of chemical and physical (autoclave) methods. Liquid infectious wastes are autoclaved prior to off-site transport and incineration. General solid waste is decontaminated by autoclave prior to off-site transport and incineration. Following decontamination by autoclave, regulated medical wastes (e.g., animal wastes, culture material, and sharps) are collected and removed for off-site transport and incineration (Roop, 1998a). LSUMC policy incorporates Federal and state hazardous waste regulations and does not permit hazardous liquid waste to be discharged into the sanitary sewer system.

Radioisotopes and radioisotope-contaminated solid and liquid wastes with half-lives less than 90 days (e.g.,  $^{32}\text{P}$  and  $^{35}\text{S}$ ) must be stored in separate containers. The packaged waste is placed into a shipping canister, and a completed Radioactive Waste Disposal Form attached. The Radiation Safety Office arranges off-site transport and land disposal of radioactive waste by a licensed contractor (LSUMC, 1998a). Radioactivity in radioactive wastes generated in the past year by LSUMC during the conduct of similar research to that proposed consisted of 0.33 microcurie ( $\mu\text{Ci}$ ) of  $^{35}\text{S}$ , 0.50  $\mu\text{Ci}$  of  $^{32}\text{P}$ , and 0.001  $\mu\text{Ci}$   $^{33}\text{P}$  (Gavin, 1998).

## 2.7 ANIMAL CARE AND USE

Prior to designing the proposed research, it was determined that there were no alternatives to using live animals to assess attenuation of, or immune responses to, *Brucella* double mutants. The number of animals needed was selected based upon the number required to achieve statistically significant results. The selection of the BALB/c mouse model allows the use of fewer mice than might be necessary with other strains. An estimated 4,120 female BALB/c mice will be required over the 4-year span of the proposed study (Roop, 1998a).

The animals will be kept in filter-bonneted micro-isolator units placed on ventilated, negative-pressure biohazard racks. The units meet BSL-3 biocontainment specifications. The investigators and personnel from LSUMC Animal Resources inventory and monitor the status of animals in the BSL-3 suite. Only authorized personnel have access to the BSL-3 suite. Access is controlled by

a key card system (Roop, 1998a). Animals are not permitted in the laboratory unless they are being used for the research (LSUMC, 1998b).

The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited LSUMC animal facilities in 1996. Criteria for AAALAC certification encompass all aspects of animal care and use, including research management, veterinary care, and physical facilities. Prior to initiating research, protocols involving animals must be approved by the LSUMC Institutional Animal Care and Use Committee. The U.S. Department of Agriculture annually inspects animal facilities at LSUMC. The facilities were in compliance in 1997 (LSUMC, 1997).

## **2.8 HUMAN HEALTH AND SAFETY**

### **2.8.1 Worker Health and Safety**

Because there is currently no vaccine available for human brucellosis, workers engaged in work with disease-causing microorganisms such as *Brucella* must be monitored to ensure that they have not become infected. In accordance with AR 385-69, a medical surveillance program has been established with the LSUMC Institutional Occupational Health Physician for workers engaged in work involving *Brucella*. The LSUMC Occupational Health Clinic directs medical monitoring of personnel working with *Brucella*. Baseline serum samples (blood samples obtained before working with *Brucella*) must be obtained and additional blood samples are obtained as deemed necessary by the LSUMC Occupational Health Physician to monitor for potential exposure. Personnel must also undergo semiannual health screening examinations as well as an examination upon termination of work with the project (LSUMC, 1997).

Maintenance workers are protected from exposure to potential pathogens by limiting access to the BSL-3 facilities. A representative from the Safety Office of Animal Resources notifies the maintenance workers of the nature of the research being conducted in the facility. During routine maintenance, all BSL-3 work ceases, work areas are disinfected, and the BSL-3 laboratory is kept vacant the night before the scheduled maintenance. Maintenance workers are provided access to personal protective equipment (LSUMC, 1997).

### **2.8.2 Public Health and Safety**

The proposed research does not involve the use of human research subjects. If a vaccine for use in humans is developed from the research, the U.S. Food and Drug Administration (FDA) will regulate its advanced development, testing, production, and use in humans. In accordance with FDA regulations, any vaccine developed will be evaluated within the context of NEPA before licensure (DA, 1996).

### **2.8.3 Accidents and Incidents**

During the 6 years in which *Brucella* work has been conducted at LSUMC, there have been three incidences of suspected exposure. The LSUMC Occupational Health Physician administered prophylactic antibiotics within 24 hours to two individuals reporting potential exposures. Neither became seropositive (developed detectable levels of antibodies indicating infection). The third

individual was diagnosed with brucellosis in 1994 and seroconversion was demonstrated. However, it was never established whether the infection was acquired within the LSUMC BSL-3 facility or from *Brucella* exposure at another facility where the individual had performed necropsies on experimentally infected goats. No laboratory exposure could be identified as having occurred at the LSUMC BSL-3 facility prior to the individual developing symptoms. The LSUMC Occupational Health Physician treated and monitored the worker. The infection was identified as being caused by a genetically altered strain of *Brucella* (Roop, 1998a).

In accordance with AR 385-69, LSUMC coordinates emergency preparedness and maintains formalized agreements with local emergency service providers. Formal agreements exist with the Shreveport Fire Department, the Public Safety Department, and the Public Health Department (Ford, 1997). The City of Shreveport has a hazardous materials (HAZMAT) team that responds to hazardous material spills. Also, the LSUMC Environmental Health and Safety Office personnel will respond with appropriate safety equipment to on-site spills of hazardous materials. The LSUMC Occupational Health Clinic is responsible for treating job injuries or exposures during regular working hours. In the event of an emergency after working hours, the LSUMC Hospital will provide emergency medical care (LSUMC, 1997).

### **3.0 ALTERNATIVES CONSIDERED**

#### **3.1 INTRODUCTION**

The proposed action and subject of this EA is a research project funded by the USAMRMC – *Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* (Alternative I, the preferred alternative). During the preparation of this EA, one alternative to the proposed action was identified. This alternative involves discontinuing plans to conduct the proposed research (Alternative II, no action).

#### **3.2 ALTERNATIVE I - CONDUCT PROPOSED RESEARCH AT LSUMC**

Alternative I entails implementing the currently planned research study as proposed to USAMRMC by LSUMC. This alternative is preferred because the proposed research activities are likely to produce important information and increased understanding of methods to prevent human brucellosis, a disease caused by *Brucella*, a potential biological warfare threat. The proposed project is the product of LSUMC investigators and is related to ongoing research at LSUMC involving immunity to *Brucella*, and as such is not transferable to another site or research group. Alternative I is the option that better meets the needs of national defense.

#### **3.3 ALTERNATIVE II - NO ACTION**

Alternative II entails discontinuing USAMRMC plans to fund the proposed study at LSUMC. This alternative is not preferred because of the need to continue research efforts toward developing a safe and effective vaccine against human brucellosis. The proposed research project has been critically reviewed by USAMRMC and determined to have the potential to advance brucellosis vaccine research. Alternative II is not preferred because it would impair national defense by disrupting research efforts directed toward protecting U.S. soldiers from *Brucella*, a potential biological warfare threat.

## **4.0 AFFECTED ENVIRONMENT**

### **4.1 INTRODUCTION**

This section of the EA describes aspects of the biophysical and socioeconomic environment that could potentially be impacted by the proposed action.

### **4.2 LAND USE AND GEOLOGY**

The proposed biomedical research will be conducted in the BSL-3 and BSL-2 containment suites at the Biomedical Research Institute at LSUMC. LSUMC lies on urban land covered by buildings, structures, roads, and parking lots. LSUMC is located on Kings Highway in the city of Shreveport in northwest Louisiana. The city of Shreveport is situated on the western bank of the Red River about 200 feet above sea level (U.S. Geological Survey, 1992). The city lies about 15 miles east of the Texas state line and 30 miles south of Arkansas. The city of Shreveport covers an area of about 115 square miles, primarily in Caddo Parish, with a small part of the city located in Bossier Parish (City of Shreveport, 1997). Caddo Parish covers an area of 603,520 acres; 575,296 acres of land and 28,224 acres of large water areas (Natural Resources Conservation Service, 1980).

Caddo Parish contains uplands, stream terraces, and the Red River alluvial plain. The uplands are located at elevations up to 465 feet above sea level; the terraces are about 160 to 220 feet above sea level. The city of Shreveport lies on the Red River alluvial plain that runs north-south in eastern Caddo Parish. The Red River lies east of the plain. A low bluff line to the west separates the plain from the Pleistocene terraces and uplands. The elevation of the alluvial plain is about 140 to 200 feet. The Red River alluvial plain and its distributaries (outflowing branches of a river) formed natural levees covered by loamy soils. The lower parts of the natural levees have clayey soils. Most sediment in the plain originated north and west of Caddo Parish, where there are older Permian red beds. Alluvial deposits of the Red River are composed of clay, silt, sand, and gravel (Natural Resources Conservation Service, 1980).

Soils in the surrounding area belong to the Woodtell-Urban land, 3% to 8% slopes, and the Guyton-Urban land complexes, of which typical areas are about 50% Guyton soils and 30% urban land. To a depth about 47 inches, Guyton soils are light brownish gray, strongly acid silt loam. The soil to a depth of about 66 inches is light gray, slightly acid, silty clay loam. Slopes are from 0% to 1%. Guyton soils are poorly drained and slowly permeable; drainage systems have lowered the seasonal high water table in some areas. In the Woodtell-Urban land complex, about 55% are Woodtell soils and 30% is urban land. The surface layer of typical Woodtell soils is dark grayish brown, medium-acid, fine sandy loam about 8 inches thick. The subsoil is red silty clay mottled in brown and gray to about 31 inches in depth. To about 47 inches, the soil layer is mottled gray, red, and yellowish brown clay. Stratified, light brownish gray sandy clay loam and sandy loam are found to about 65 inches. The soils drain poorly and have high shrink-swell potential (Natural Resources Conservation Service, 1980).

### **4.3 CLIMATE AND AIR QUALITY**

Caddo Parish has a subtropical climate characterized by warm temperatures, relatively long frost-free season, little significant snowfall, high humidity, abundant precipitation, and infrequent damaging winds. Warm, moist, maritime tropical air from the Gulf of Mexico strongly influences the climate. Cold fronts consisting of continental polar air from Canada occur briefly but frequently in winter and spring. Maximum precipitation occurs during the winter and spring. Precipitation in summer usually takes the form of widely scattered thunderstorms. In summer and late autumn, Caddo Parish may experience heavy showers and rains associated with tropical storms from the Gulf of Mexico (Natural Resources Conservation Service, 1980). The annual average temperature in the city of Shreveport is 65.2°F. The average high and low temperatures range from 76.2°F to 54.2°F. Average annual precipitation is 46.11 inches. The average annual snowfall of 1.7 inches occurs between December and March. The average relative humidity is 88% in the morning and 58% in the afternoon (National Weather Service, 1993).

In 1996, Shreveport was in attainment for ozone (O<sub>3</sub>), sulfur dioxide (SO<sub>2</sub>), and particulate matter greater than 10 microns in diameter (PM<sub>10</sub>) (LDEQ Air Quality Division, 1996). The state of Louisiana was in attainment for nitrogen dioxide (NO<sub>2</sub>), carbon monoxide (CO), and lead (Pb) in 1996. Data on emissions of volatile organic compounds (VOCs) show a 70% reduction since monitoring began in 1975. The pollutant standards index (PSI) is computed for four Louisiana cities, including Shreveport. In 1995, the air quality of Shreveport was in the good-to-moderate range. Based on weekly sampling of air toxic pollutants from July to December 1997, Shreveport met the Louisiana Air Toxic Ambient Air Standards (LDEQ, 1997).

Indoor air quality is monitored for the protection of human health. The main air-handling system for the Biomedical Research Institute was sampled for chemical and radiation contaminants in 1997. No radiologic or VOC contamination was detected. The Biomedical Research Institute fume hood exhaust system showed no radioactive contamination on July 25, 1997 (Gavin, 1998).

### **4.4 WATER RESOURCES AND WETLANDS**

The Red River originates in eastern New Mexico, then flows across parts of Texas, Oklahoma, and Arkansas and into northwestern Louisiana. At Shreveport, the river flows southeast to the Atchafalaya River. From Arkansas to Alexandria, Louisiana the banks of the Red River are high 20- to 35-feet above low-water level. Shreveport lies within the Red River Basin (LDEQ, 1998). In Caddo Parish, major distributaries of the Red River and its alluvial plain cover almost one-third of the total area. Some major distributaries are inactive. The principal sources of surface water for the parish have been the Red River, Caddo Lake, Cross Lake, and Wallace Lake. The Red River drains 56,000 square miles and is the largest source of surface water. Cross Lake is the source of drinking water for the City of Shreveport (Natural Resources Conservation Service, 1980). The Shreveport-Bossier City Metropolitan Area crosses 11 watersheds, including Bayou Pierre and Cross Bayou. The Red River flows through both of these watersheds (USEPA, 1998). The Shreveport Department of Water and Sewerage supplies water to LSUMC. The Medical School uses approximately 48,000,000 gal of water per year. Based upon a square footage comparison with the Medical School, water usage for the facilities in which the proposed action will be conducted is estimated at 317,000 gal annually.

Groundwater in Caddo Parish is supplied by the Red River alluvial aquifer. The alluvial aquifer underlying the flood plains of the Red River valley is of the Pleistocene and Holocene age, and consists of upward sequences of gravel, sand, silt, and clay. Sand is more fine-grained in the top layers, with coarser sand and gravel in deeper layers. The aquifer recharges from infiltration of rainfall, from adjacent aquifers, and from stream flooding (LDEQ, 1998).

There is currently no National Wetlands Inventory (NWI) map available for the Shreveport area. When the wetlands were being mapped by the NWI, most of the Shreveport area was in pine plantation, with some palmetto in the understory. Some Guyton soils were hydric. The NWI considered some concave areas of the Guyton soils as wetlands (Storrs, 1998).

#### **4.5 PLANT AND ANIMAL ECOLOGY**

Caddo Parish is inhabited by game animals, including deer, cottontail and swamp rabbits, gray and fox squirrels, ducks, quail, and doves. Other animals in the area include beaver, mink, raccoon, foxes, skunk, nutria, opossum, bobcat, and otter. The Red River is along the migration routes for many species of birds. The wood duck lives around the lakes and bayous all year. Black and white crappie, bluegills, white and largemouth bass, redear sunfish, channel catfish, and pickerel are found in the parish lakes (Natural Resources Conservation Service, 1980). There are no state or federal parks, scenic streams, or wildlife refuges or management areas in the area of LSUMC. The Louisiana Department of Wildlife and Fisheries reports no rare, threatened, or endangered species or any critical habitats in the LSUMC area (Tarver, 1998).

#### **4.6 HISTORICAL AND CULTURAL RESOURCES**

The Louisiana Office of Cultural Development Division of Archaeology has determined that significant archaeological or historic resources are not located near LSUMC (Hobdy, 1998).

#### **4.7 ENERGY RESOURCES**

Southwestern Electric Power provides electricity, and ARKLA Gas supplies natural gas to LSUMC. LSUMC used 55,238,400 kilowatt hours (kWh) of electricity and 1,837,592 cubic feet (ccf) of natural gas between July 1996 and June 1997. Energy consumption for the BSL-3 suite and for Room 2-349 is based on estimated area. Annual energy usage for the BSL-3 suite based on an estimated area of 1,600 square feet is 66,240 kWh of electricity and 2,203.2 ccf of natural gas. Energy consumption for Room 2-349, based on about 1,000 square feet of area, is 41,401 kWh of electricity and 1,377 ccf of natural gas (Gavin, 1998).

#### **4.8 SOCIOECONOMIC ENVIRONMENT**

According to the 1990 U.S. Census, the population of Caddo Parish was 248,253, a decrease of 4,105 from 1980. The population of the city of Shreveport was 198,528 in 1990. The population was 54% white, 45% black, and <1% American Indian, Eskimo, Aleut, Asian, or Pacific Islander. In 1990, 1% of the population was of Hispanic origin. In the Shreveport Metropolitan Statistical Area, 80.6% of the population was urban and 19.4% was rural. Of persons age 25 years or older, 17.5% had earned a bachelor's degree or higher. In 1990, 22% of all persons and 17.4% of all

families within the Shreveport Metropolitan Statistical Area were living below the poverty level (U.S. Census, 1990).

LSUMC serves Shreveport, Bossier City, and the surrounding area. LSUMC employs about 4,300 people, including more than 290 full-time and 100 part-time faculty (LSUMC, 1997). The Biomedical Research Institute employs about 200 scientists, technicians, and support personnel (Biomedical Research Foundation of Northwest Louisiana, 1996).

#### **4.9 TRANSPORTATION**

LSUMC is located southwest of downtown Shreveport on Kings Highway, which runs east-west. By automobile, LSUMC is accessible from I-49, which runs north-south, by exiting at Kings Highway. U.S. Route 171 runs north-south and intersects Kings Highway west of LSUMC. Major airlines serve the Shreveport Regional Airport, located southwest of LSUMC on I-20. From the airport the campus is accessible by automobile by taking I-20 East to I-49 South. The Shreveport Downtown Airport is located northeast of LSUMC. SporTran City Transit System, the public transportation system, serves Shreveport and Bossier City across the Red River. Greyhound/Trailways Lines provides bus service to the area (Shreveport-Bossier Convention & Tourist Bureau, 1998).

## **5.0 ENVIRONMENTAL CONSEQUENCES**

### **5.1 INTRODUCTION**

In this section, the potential for significant environmental impacts (direct, indirect, and cumulative) to result from the proposed research will be discussed. This discussion will identify cause and effect relationships between the proposed action and impacts to the environment, including impacts that may not necessarily occur but are reasonable. The term “consequence” refers to the outcome of an event or events without considering probability. Where possible, potential events will be characterized in terms of both their potential consequence and the probability (likeliness) that they will occur.

### **5.2 ENVIRONMENTAL CONSEQUENCES**

#### **5.2.1 Land Use and Geology**

It is highly unlikely that the proposed research project (Alternative I) would impact land-use patterns, geology, or soils at LSUMC, or within Shreveport. All proposed activities will be conducted in existing facilities that have been sited in conformance to local topography. It is estimated that the quantity of wastes generated from the proposed research project will be negligible when compared to wastes generated from all of LSUMC. The portion of wastes disposed of in local landfills will likely also be a negligible component of the total wastes from all of LSUMC. Because construction is neither planned nor anticipated, no disruption of land-use patterns or geological resources is likely. Implementing Alternative II (no action) would eliminate any negligible impacts to land-use patterns, soils, or geological resources.

#### **5.2.2 Climate and Air Quality**

It is highly unlikely that negative impacts to air quality will result from the conduct of the proposed study (Alternative I). Impacts to air quality will result from the incineration of regulated medical wastes and the contribution of on-road mobile sources of air pollution (trucks and automobiles) transporting employees and providing services in support of the proposed research. The contributions of these impacts to regional air quality are likely to be negligible in comparison to those of other activities in the area. Current regional air quality is good (see Section 4.3). Implementing Alternative II (no action) would eliminate the negligible impacts associated with conducting the proposed action.

#### **5.2.3 Water Resources and Wetlands**

Implementation of the proposed action is unlikely to significantly impact water resources near LSUMC or in the Shreveport area. Quantitatively, wastewater contributions expected from conducting the proposed study are likely to be negligible (1,000 gal annually) compared with total wastewater discharges resulting from LSUMC (400,000 gal daily). It is estimated that the amount of wastewater generated by the proposed research is about 0.007% of LSUMC wastewater contributions. Wastewater generated by all LSUMC activities is approximately 146 million gals annually. Wastewater discharged to the sanitary sewer system flows from LSUMC to

the Lucas Wastewater Treatment Plant in the City of Shreveport. Potentially contaminated wastewater generated in the BSL-3 facility must be rendered noninfectious prior to discharge to the sanitary sewer system. Hazardous chemical waste, regulated medical waste, and radiologic waste generated by the proposed action must be segregated when generated. Adherence to Federal and state law and LSUMC policy governing waste disposal further mitigates impacts to surface water resources. Wastewater analyses conducted on January 20, 1998 indicate that LSUMC discharges comply with the city of Shreveport ordinances (Gavin, 1998). Implementing Alternative II (no action) would eliminate the negligible impacts associated with implementing the proposed action.

Adverse impacts to wetlands from implementing the proposed action (Alternative I) are highly unlikely. The proposed action will be conducted in existing facilities and no construction is planned or anticipated, and therefore stormwater runoff patterns will not be impacted. Wastewater will not be discharged to wetlands. Implementation of Alternative II (no action) would eliminate potential impacts associated with the proposed action.

#### **5.2.4 Plant and Animal Ecology**

It is highly unlikely that adverse impacts to plant or animal ecology will result from the conduct of the proposed study (Alternative I). No construction or renovation is planned that could impact plant or animal habitat. *Brucella* does not cause plant disease. Impacts to animals near the LSUMC facilities in which *Brucella* research will be conducted are highly unlikely. The facilities in which mice will be housed have features that nearly eliminate the likelihood of animal escape. In the unlikely event that a mouse would escape it would be unlikely to survive in the natural environment. Should a mouse escape and survive, its ability to transmit *Brucella* while alive is limited. A potential adverse impact (brucellosis) to susceptible native mammalian species would be possible from consumption of *Brucella*-infected mice in the unlikely event that they escaped. Alternative II (no action) would eliminate any potential adverse impacts to local plant and animal ecology.

#### **5.2.5 Historical and Cultural Resources**

Adverse impacts to historical and cultural resources are unlikely to result from implementation of the proposed alternative. No renovations or construction are planned that would negatively impact existing resources. The proposed research will be conducted indoors in existing facilities. Significant archaeological or historic resources do not exist near LSUMC (Hobdy, 1998). Implementing Alternative II (no action) would eliminate any potential for adverse impacts on historical or cultural resources.

#### **5.2.6 Energy Resources**

Adverse impacts to energy resources are unlikely to result from implementing the proposed action. The proposed research will be conducted in existing facilities in which similar activities are currently conducted. The proposed action is not anticipated to alter existing resource utilization. Implementing Alternative II (no action) would eliminate these negligible impacts on energy resources.

### **5.2.7 Socioeconomic Environment and Aesthetics**

Implementation of the proposed action (Alternative I) will likely result in minor positive impacts on the local economy through employment and local purchases. Potential exists for substantial positive benefits to the U.S. through future advanced development of a human brucellosis vaccine. Economic impacts are also possible through the application of research findings that improve and protect animal and/or human health. Significant impacts from noise or odors are not anticipated. Similar activities have been conducted at the proposed research facilities without observed impacts or complaints. Implementing Alternative II (no action) would eliminate the minor positive impacts to the local economy likely to result from implementing the proposed action and the potential for substantial positive impacts to national defense.

### **5.2.8 Transportation**

Implementation of the proposed action (Alternative I) will likely have negligible or no impact on transportation resources. There is no construction or renovation planned that would alter existing traffic patterns. Impacts resulting from the commuting of the six personnel involved in implementing the proposed study will be negligible. Implementation of Alternative II (no action) will eliminate these negligible impacts to transportation resources associated with implementing the proposed action.

### **5.2.9 Public Opinion**

Public opinion has been an issue in the conduct of biological warfare defense research and development activities and was extensively discussed in the BDRP FPEIS. There is strong congressional and public support for DoD policy to provide service men and women with the best possible protection against potential biological warfare agents. Potential criticisms, however, include the perceived potential for this research to be used for offensive purposes, the efficacy of biological defense vaccines, distrust of the military, and whether the military should be involved in vaccine development. Some public concerns relate to the existence of biological defense programs *per se*; others, to the intent, need for, and benefits of such programs. Some of the other concerns are specific to the impacts of actions, such as the use of animals in research and the use and handling of recombinant DNA technology. Issues such as these are not unique to the proposed research but are concerns associated with vaccine and/or other biomedical research and development activities in general.

The government and facilities supported by the government (e.g., LSUMC) do not engage in work related to the production or use of offensive biological weapons as required by the *Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological (Biological) and Toxin Weapons and on their Destruction* (the Biological Weapons Convention of 1972) to which the U.S. is a signatory.

The BDRP FPEIS examined the use of recombinant DNA technology and concluded that significant issues associated with its use were related to the existence of the biological defense program rather than to specific sites that were analyzed. The analysis performed in the BDRP FPEIS identified no actual significant adverse impacts resulting from the use of recombinant DNA

technology. This conclusion was validated by subsequent biological defense research site-specific assessments.

### **5.2.10 Human Health and Safety**

The proposed research project at LSUMC involves using *Brucella* species capable of causing human disease. Although rarely fatal, brucellosis can result in chronic adverse health effects if left untreated. The prompt administration of antibiotic therapy following known or suspected exposure has been effective in preventing acute and/or chronic disease.

#### *5.2.10.1 Worker Health and Safety*

There are risks to those working with disease-causing microorganisms such as *Brucella*. Most accidents that have occurred in the past resulted from techniques no longer acceptable (e.g., mouth pipetting) and from the unknowing and unintentional generation of aerosols containing infectious particles. Risks to laboratory workers have decreased over time through the implementation of improved biosafety practices, equipment, and facilities (DA, 1989). The risk to workers of laboratory-acquired infections from the conduct of the proposed study (Alternative I) is minimized by implementing the environmental engineering and work practice controls described in the CDC/NIH guidelines (1993), AR 385-69, DA Pamphlet 385-69, and in LSUMC SOPs. Engineering controls are in place in the LSUMC BSL-3 laboratory to prevent *Brucella* organisms from contaminating the laboratory environment. Risk of exposure is further mitigated by the use of required laboratory work practices designed to reduce the likelihood of aerosol production during routine activities. Work practice controls used to prevent contamination of environments external to the BSL-3 laboratory include disinfecting work surfaces, floors, and drains and the segregation and autoclaving of waste materials, work clothes, and other material prior to removal from containment facilities. In addition to the use of engineering and work practice controls to reduce the risk of exposure to *Brucella*, regular monitoring of worker health is required. Antibiotic therapy must be administered to workers with possible exposures. Adverse impacts to worker health resulting from similar work conducted at LSUMC have not been observed. While there have been potential exposures to *Brucella*, there has been only one incident of confirmed disease and that was successfully resolved upon treatment by the LSUMC Occupational Health Physician. The source of that infection is uncertain (Roop, 1998a) (see Section 2.8.3). Implementing Alternative II (no action) would eliminate the potential for adverse impact to worker health and safety associated with the conduct of the proposed study.

#### *5.2.10.2 Public Health and Safety*

The risk to public health from the conduct of *Brucella* research is negligible. Because of the redundant safety features required of BSL-3 facilities, it is unlikely that the public would be exposed to viable *Brucella* originating from the LSUMC laboratory. Adherence to Federal and state regulations pertaining to the safe handling and disposal of hazardous chemicals, radioisotopes, and potentially infectious material further mitigates the likelihood of impact to public health and safety. Similar work has been performed at LSUMC without observed impacts to public health. Implementing Alternative II (no action) would eliminate the minimal potential

for adverse impact to public health and safety associated with the conduct of the proposed study and the potential for positive impact to public health from developing a *Brucella* vaccine.

### 5.2.10.3 Accidents and Incidents

A Maximum Credible Event (MCE) has been developed for the proposed research that examines a realistic worst-case scenario and its potential to infect personnel within the laboratory and the surrounding community. The scenario involves a worker accidentally spilling the contents of two, 1-liter flasks each containing 200 milliliters (ml) of *Brucella melitensis* culture. The accident occurs in a 2,250-square-foot (51,750 liters air volume) laboratory within the BSL-3 facility. The 400 ml of culture that would be spilled contains approximately  $1 \times 10^9$  cfu/ml (or  $400 \times 10^9$  total organisms)  $40 \times 10^9$  infectious doses, assuming that the infectious dose is 10 cfu (Kaufmann, 1995; Kaufmann and Boyce, 1995). This MCE analysis assumes that 0.1% of the spilled solution is aerosolized and that 99% of the aerosolized spill would settle as droplets within 30 minutes. This results in  $4.0 \times 10^5$  infectious doses remaining aerosolized in the laboratory. Assuming 95% of this aerosol is vented,  $3.8 \times 10^5$  of the  $4.0 \times 10^5$  aerosolized infectious human doses would be vented through the exhaust stack on the building roof. HEPA filtration, such as is required of biological safety cabinets in the BSL-3 laboratory, is not required on the exhaust stack. The exhaust stack measures 185 feet from the ground to the top of the stack and 20 feet from the roof to the top of the stack. Using plume dispersion modeling (see DA, 1989), fewer than 800 infectious doses per liter of air would remain at less than 2 meters from the stack and would further dilute to fewer than 80 infectious doses per liter at 7 meters. This level of contamination would not pose a significant risk to the community (see DA, 1989). The risk to the public from vented organisms would be further reduced by the destruction of *Brucella* by ultraviolet radiation from the sun (Roop, 1998b).

The worker spilling the flasks and laboratory workers in close proximity are at greatest risk of infection (14,137 infectious doses per liter of air). At this concentration, a worker and any coworker coming immediately to his/her aid not wearing respiratory protection might inhale  $2.1 \times 10^5$  infectious doses. A worker exposed in this manner would receive prophylactic therapy with antibiotic(s) (e.g., doxycycline) and seromonitoring. The *Brucella* used in the proposed study are sensitive to the tetracyclines (a family of antibiotics). Introduction of genes conferring tetracycline resistance to *Brucella* is prohibited by Federal law and institutional policy. SOPs detail the protocols for safely containing and decontaminating *Brucella* spills (Roop, 1998b).

The LSUMC facilities are designed to contain *Brucella*, and work is conducted under SOPs that implement CDC/NIH guidelines and DA regulations. Adherence to these procedures will minimize risks to workers and the community.

### 5.2.11 Environmental Justice

Executive Order 12898, *Federal Actions to Address Environmental Justice in Minority and Low Income Populations*, requires Federal agencies to consider whether their projects will result in disproportionate adverse impacts on minority or low-income populations. The U.S. Census defines the poverty level as the income level, based on family size, age of householder, and the number of children under 18 years of age, that is considered too low to meet essential living

requirements without regard to the local cost of living. The U.S. Census considers a poverty area as an area in which at least 20% of the population lives below the poverty level. Implementation of the proposed action (Alternative I) is highly unlikely to result in significant adverse impacts to the human environment, including human health, and thus no disproportionate significant adverse impacts on minority or low-income populations. Implementing Alternative II (no action) would eliminate the potential for adverse impacts.

### **5.3 CUMULATIVE IMPACTS**

The CEQ regulations implementing NEPA define cumulative impacts to the environment as those effects resulting from the impact of the proposed action when combined with past, present, and future actions (40 CFR Part 1508.7). Thus, cumulative impacts are the sum of all direct and indirect impacts, both adverse and positive, that result from the incremental impacts of the action when added to other past, present, and reasonably predictable future actions regardless of source. Cumulative impacts may be accrued over time and/or impacts in conjunction with other pre-existing effects from other activities in the area (40 CFR Part 1508.25).

No negative cumulative environmental impacts have been observed from the conduct of activities similar to the proposed action at LSUMC. It is highly unlikely that cumulative adverse environmental impacts will result from conducting the proposed research study (Alternative I). Contributions of the proposed study to the LSUMC waste stream or resource utilization are negligible. The proposed research will be conducted in existing facilities and no construction or renovations are planned. Implementing Alternative II (no action) will eliminate the negligible adverse cumulative impacts associated with implementing the proposed action.

### **5.4 COMPARISON OF THE PROPOSED ACTION WITH THE ALTERNATIVES**

#### **5.4.1 Alternative I - Conduct Proposed Research at LSUMC**

The research methods, hazardous materials, safety, and containment practices employed in the conduct of the proposed research study for USAMRMC at LSUMC are consistent with those required and employed at other biomedical research institutions performing similar work. The potential for adverse impacts to the human environment resulting from the conduct of the proposed research is negligible and is mitigated by adherence to existing regulations and guidelines developed to protect human health and the environment. Positive impacts to U.S. civilian and military are likely.

#### **5.4.2 Alternative II - No Action**

Alternative II, no-action, involves not conducting the proposed research at LSUMC as submitted and selected for funding by the USAMRMC. Implementing this alternative would eliminate the potential negligible adverse impacts associated with the proposed action. This alternative is not preferred, however, because it would also eliminate the potential positive impacts associated with progress toward developing a safe and effective vaccine against human brucellosis.

## 6.0 CONCLUSIONS

The principal conclusions of this EA are: (1) the conduct of the proposed research project—*Identification of Secondary Mutations Which Enhance and Stabilize the Attenuation of Brucella htrA Mutants: Improving Brucella htrA-based Strains as Vaccine Candidates* (Alternative I, the preferred alternative) is not expected to result in significant adverse environmental impacts; (2) implementation of the preferred alternative will likely result in important benefits to the U.S. by enhancing progress toward developing an acceptable vaccine against human brucellosis; and (3) ceasing the proposed research project (Alternative II - no action) will eliminate the negligible environmental impacts associated with conducting the research but will also eliminate potentially significant advances in brucellosis vaccine development.

Laboratory work involving *Brucella* is currently being conducted at LSUMC without significant environmental impact. The most severe potential effects associated with the proposed *Brucella* research are predicted to be negligible, and to date, all observed effects have been insignificant. Potential risks to human health and the environment will continue to be mitigated by applying required standards, practices, and controls pertaining to the safe use and disposal of hazardous biological and chemical materials; the protection and conservation of natural resources; and the safe and ethical conduct of studies requiring animal subjects.

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## 10.0 ACRONYMS AND ABBREVIATIONS

μCi	microcurie
AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AAPSO	Army Acquisition Pollution Prevention Support Office
AR	Army Regulation
ATSDR	Agency for Toxic Substances & Disease Registry
AWQC	Ambient Water Quality Criteria
BDRP	Biological Defense Research Program
BSL	biosafety level
CAA	Clean Air Act
ccf	cubic feet
CDC	Centers for Disease Control & Prevention
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations
cfu	colony forming units
CHP	Chemical Hygiene Plan
CO	carbon monoxide
CO <sub>2</sub>	carbon dioxide
DA	Department of the Army
DNA	deoxyribonucleic acid
DoD	Department of Defense
EA	Environmental Assessment
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FPEIS	Final Programmatic Environmental Impact Statement
gal	gallons
GEM	genetically engineered microorganisms
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HAZMAT	hazardous material
HEPA	high-efficiency particulate air
HOCl	hypochlorous acid
IACUC	Institute for Animal Care and Use Committee
IBC	Institutional Biosafety Committee
KWh	kilowatt hours
LSUMC	Louisiana State University Medical Center
MCE	Maximum Credible Event
MCL	Maximum Contaminant Level
mg/L	milligrams per Liter
ml	milliliters
MSDS	Material Safety Data Sheets
msl	mean sea level
NAAQS	National Ambient Air Quality Standards
NEPA	National Environmental Policy Act
NIH	National Institutes of Health

NO	nitric oxide
NO <sub>2</sub>	nitrogen dioxide
NPDES	National Pollutant Discharge Elimination System
NRC	Nuclear Regulatory Commission
NWI	National Wetlands Inventory
O <sub>2</sub> <sup>-</sup>	superoxide radical
O <sub>3</sub>	ozone
OSHA	Occupational Safety & Health Administration
Pb	lead
PM10	Respirable particulate matter
ppm	parts per million
psi	pounds per square inch
PSI	Pollutant Standards Index
RCRA	Resource Conservation & Recovery Act
REC	Record of Environmental Consideration
ROD	Record of Decision
SO <sub>2</sub>	sulfur dioxide
SOP	Standard Operating Procedures
SVOCs	semi-volatile organic compounds
USAMRMC	U.S. Army Medical Research & Materiel Command
USC	U.S. Code
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
VOCs	volatile organic compound